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Aquatic microdiversity from urban cenotes in Cancun, Quintana Roo, Mexico

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Abstract

The microdiversity of cenotes in the Yucatan Peninsula, Mexico has been little studied, with the phytoplankton and protists being the most representative species. However, all previous studies have been focused on cenotes associated with touristic activities, leaving a gap in the understanding of cenotes located within urban areas. The present study is dedicated to the identification of phytoplankton and protists in the cenotes of Cancun, Quintana Roo, Mexico. We conducted our research in four urban cenotes, collecting samples using a 150 µm plankton net, filtering them with a 45 µm membrane, and examining them under optical microscopy. Subsequently, we calculated the abundance, richness, and completeness of the samples. Our findings revealed a total of 6 phyla, 4 subphyla, 10 classes, 8 subclasses, 15 orders, 15 families, 18 genus, and 17 species and 4 species indeterminata in the cenotes of Cancun, Quintana Roo, Mexico. Among these, there were 8 species of phytoplankton and 1 species indeterminata, while 9 species of protists and 3 species indeterminata. These results highlight the remarkable species richness and the complex structure and composition of urban cenotes, suggesting that some species may be unique to this particular ecosystem. Currently, there is limited knowledge regarding the behavior of these aquifers (urban cenotes), and a comprehensive inventory or characterization of their microdiversity is lacking. Such information could be instrumental in the management, conservation, and sustainable use of these valuable aquifers.

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Keywords
Microdiversity, phytoplankton, protists, urban cenotes

Introduction

Cancun is located in the northern part of the Yucatan Peninsula within the state of Quintana Roo, Mexico. The karst relief of the Yucatan Peninsula is formed by depressions, sinkholes, and caverns. Occasionally, some of these caverns collapse, producing "cenotes," a word of Mayan origin ("*ts' ono 'ot or*" "*d'zonot*") that means "cave with a deep pool", referring to any location with accessible groundwater (Back 1985). Rainwater infiltrates and accumulates in the subsoil of the karst, creating a column of fresh water that rests atop a denser saline water mass resulting from natural seawater intrusion. The contact between these two water masses, the freshwater and marine, forms a mixing zone known as a halocline. This freshwater mass constitutes the only source of freshwater within the Yucatan Peninsula. The formation of cenotes is a consequence of the karst process occurring within the peninsula, resulting from a complex sequence of events. It starts from a flooded cave, and a grotto or a pitcher-type cenote can be formed by the collapse or partial collapse of the roof. Subsequently, the complete collapse of the ceiling results in the formation of a cylindrical cenote. From the cylindrical cenote, an aguada-type cenote may evolve due to sedimentation and the gradual subsidence of the adjacent area (Fig. 1) (Gaona-Vizcayno et al. 1980; Schmitter-Soto et al. 2002a).

The karst relief of the Yucatan Peninsula provides the environmental conditions, and the aquifer's unique characteristics contribute to the formation of a distinctive ecosystem primarily reliant on microbiome activity (Back 1995; Batllori-Sampedro et al. 2012). Microbiological components within cenotes are throughout the water

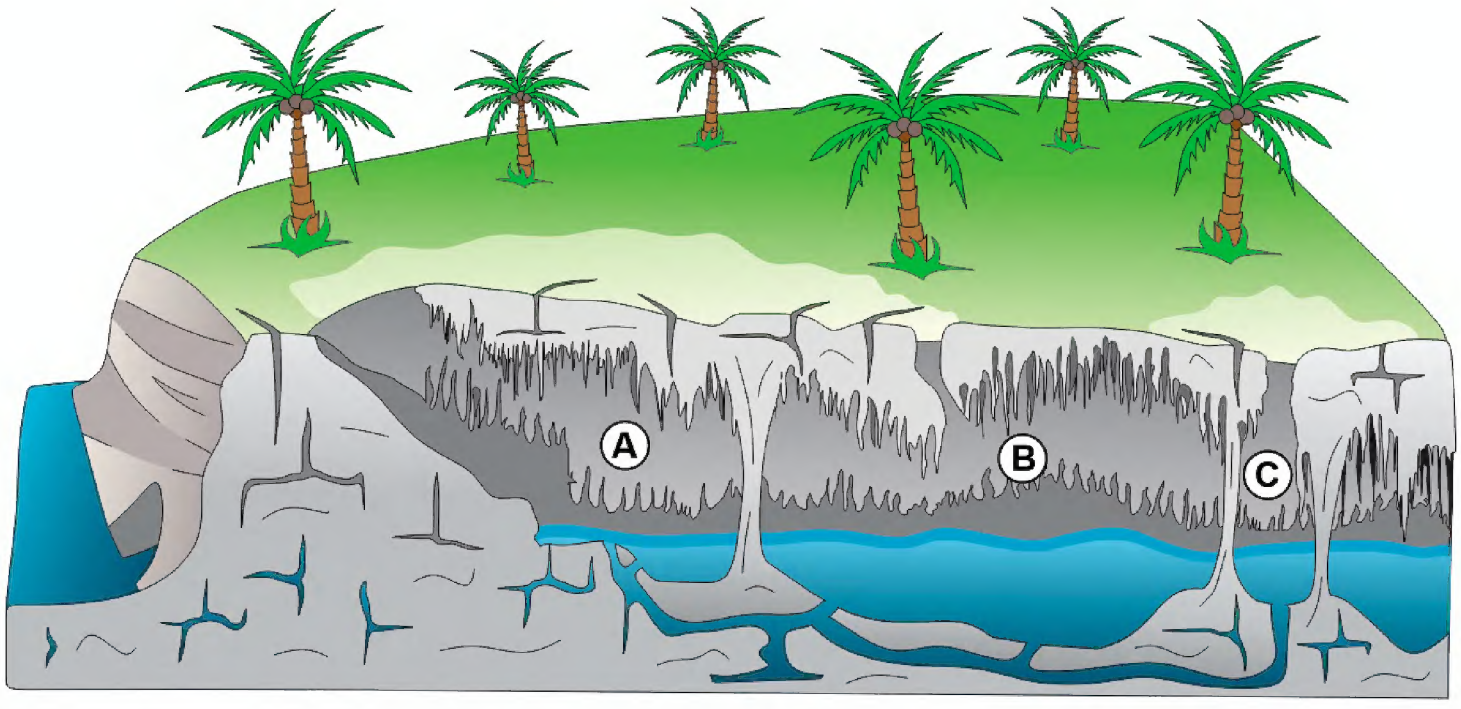

Figure 1. Types of cenotes in the Yucatan Peninsula, Mexico **A** cavern **B** grotto or pitcher-type, and **C** cylindrical cenote.

column, predominantly near the surface. However, the biodiversity of phytoplankton and protists in both fresh and marine waters in the Caribbean region of Mexico has remained unexplored. Indeed, there is a substantial knowledge gap concerning these aquatic systems and their phytoplankton and protists communities (Álvarez-Cadena et al. 2007). Only a few studies have delved into the biodiversity within cenotes, particularly focusing on phytoplankton or cyanobacteria (Arana-Ravell et al. 2019; Moore et al. 2019), while others have explored microbial diversity in cenote sediments and the water columns (Schmitter-Soto et al. 2002b; Suárez-Moo et al. 2022). These investigations have identified species associated with various phyla, including Bacillariophyta, Cryptophyta, Chlorophyta, Chrysophyta, Euglenophyta, Pyrrophyta, Xanthopyta, Dinophyta and the most dominant Cyanobacteria. Some of the orders presented in the cenotes are Synechococcales, Chroococcales, Oscillatoriales, Nostocales, Spirulinales, Pleuroscapsales and Chroococcidiopsidales. Representative genus such as *Flavobacterium*, *Prochlorococcus*, *Brevundimonas*, *Rhodobacter*, *Novosphingobium*, *Desulfobacterium*, *Acinetobacter*, *Pseudomonas*, *Chroococcus*, *Tetrastrum*, *Cryptomonas*, *Encyonopsis*, *Pseudanabaena*, *Aphanocapsa*, *Epigloeosphaera*, *Monoraphidium*, *Brachysira*, *Encyonopsis*, among others, have been commonly observed in these cenote ecosystems.

Each study performed in the cenotes of Yucatan Peninsula, Mexico, with a focus on microbial diversity, has consistently revealed a significant number of species within the sampled sites, along with the variations in the abundance and presence of species between the rainy and dry seasons. However, it is essential to note that all of these prior investigations were conducted in cenotes associated with tourist activities, such as swimming or diving, or in cenotes situated in rural areas or dense jungles. The present study is focused on cenotes located within urban settings, such as Cancun, Quintana Roo, Mexico. These urban cenotes are surrounded by residential units and roadways, presenting a distinctive ecological context.

Urban cenotes, unlike their counterparts in more pristine environments, lack a specific designated purpose. Some of these cenotes are situated within public parks, nestled between bustling avenues, or located on private properties. Only a handful of them are suitable for swimming, while many suffer from issues like litter, discarded tires, and even electrical waste contamination. Despite these significant challenges, research on urban remains limited. Microbial diversity within these cenotes holds particular importance, as it serves as a natural bioindicator of eutrophication and environmental impact. Cell abundance in these ecosystems is influenced by various factors, including biological factors, nutrient levels, organic matter content, pH, mineralization, and more (Darley 1987; Livingston 2001). However, due to the absence of comprehensive data, distinguish between endemic and foreign species within these ecosystems remains a challenge. Furthermore, identifying which species can effectively serve as bioindicators of environmental impact or eutrophication is currently beyond our reach. This study aims to address these knowledge gaps by exploring species richness, community structure, and composition in urban cenotes, with a particular focus on phytoplankton and protists. The data generated through this research can play a crucial role in guiding the management, conservation, and sustainable use of these unique and vulnerable ecosystems.

Methods

Study area

Cancun is located within the municipality of Benito Juárez in the state of Quintana Roo, Mexico (Fig. 2A). Its precise geographical coordinates are 21°09.41'N, 86°49.29'W. The topography of the region is notably flat, with elevations seldom exceeding 10 m above sea level. The predominant vegetation type is a subdeciduous tropical forest, and the area boast an average annual temperature of 25 °C (Rzedowski 2006). For the study we collected samples from four cenotes located within the city of Cancun (Fig. 2B), designated as C1 (a mix between cavern and aguada-type cenote), C2 (a grotto or a pitcher-type cenote), C3 (an aguada-type cenote), and C4 (an aguada-type cenote), corresponding to cenotes 1–4, respectively (Suppl. material 1).

Sample collection

Sample collection was conducted during two distinct seasons of the year: the rainy season, spanning from September to December, characterized by higher rainfall rates; and the dry season, occurring between April and July, marked by reduced precipitation, elevated temperatures, and increased evaporation. The sampling campaign commenced in September 2017 and concluded in July 2018. At each sampling station, two separate water samples were obtained. Each sample consisted of 10 liters of water, which underwent filtration using a 150 µm plankton net (Aquatic Biotechnology, 40cmØx233cmL CP3-110). Subsequently, the samples were suspended in 300 mL of water sourced from the same cenote and stored in sterile 500 ml screw-cap bottles at room temperature. One of the pared samples was fixed using a 4% Lugol solution, while the other was analyzed in its fresh state, following a modified method by Delgado and Sánchez (2006).

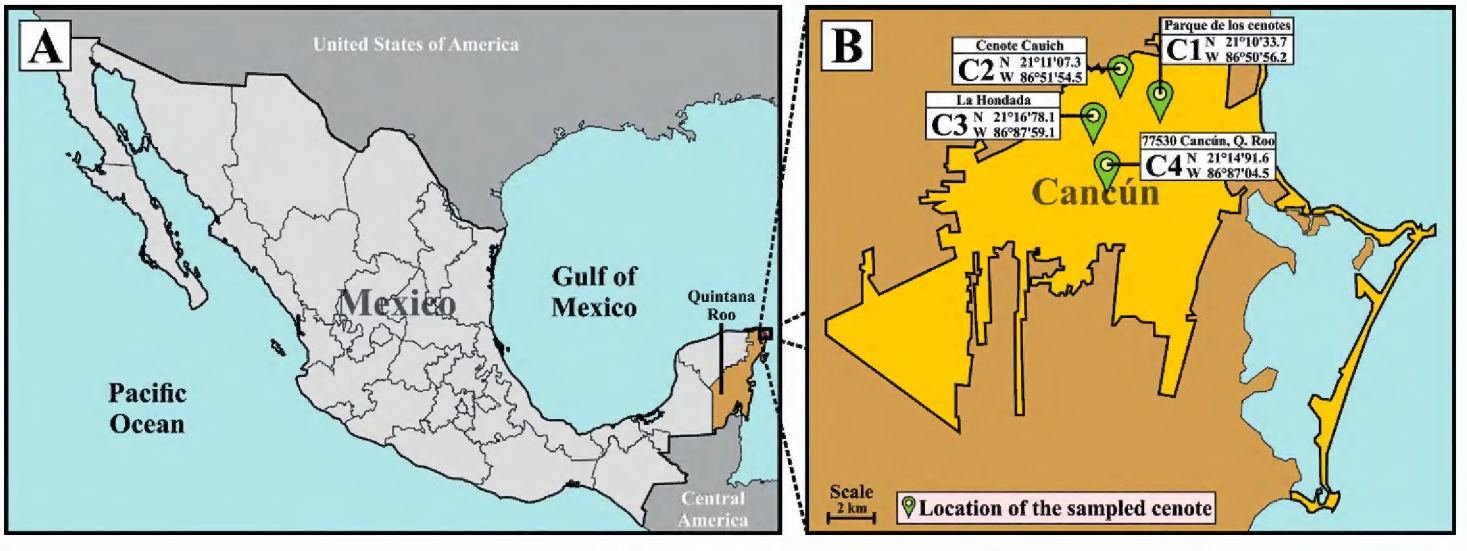

Figure 2. Sampled cenotes in Cancun, Quintana Roo, Mexico **A** Mexico map highlighting Quintana Roo in light brown, with a small box indicating the location of Cancun **B** Cancun map in yellow with green location markers representing the global coordinates of the sampled cenotes: C1 (Parque de los cenotes), C2 (Cenote Cauich), C3 (La Hondada), and C4 (77530 Cancun, Quintana Roo).

Sample processing, taxonomic identification and quantification method

After collection, the samples were stored at room temperature for a maximum of 24 hours. Subsequently, they were filtered through a sterile test tube using a Buchner funnel containing a 45 μm membrane (Millipore). The material trapped on the membrane was resuspended in 1 ml of distilled water for immediate observation as soon as possible. For analysis, taxonomic identification, and documentation, the samples were examined under an optical microscope (Motic and Labomed) with the 10×, 40×, and 100× objectives. Images were recorded using a Sony camera. Each microorganism was identified using established taxonomic keys (Luna-Pabello 2006; Barrios-Barcia and Puig-Infante 2012; Sigala-Regalado et al. 2016; Quiroz-González and Rivas-Acuña 2017; Guiry and Guiry 2020; WoRMS 2020; Siemensma 2023).

To determine the number or organisms, the following equation was applied:

$$\frac{N}{L} = \frac{((C)(v1))}{((v2)(v3))}$$

where N is the total number of organisms, C denotes the number of organisms that were quantified, v1 signifies the volume that was concentrated, v2 indicates the volume used for counting, and v3 represents the volume that was sampled (Gómez-Márquez et al. 2013). The results are expressed as the number of organisms per liter (L) or milliliter (mL).

Completeness analysis

To assess the completeness of the species inventory for each cenote, we employed species accumulation curves as proposed by Colwell and Coddington (1994). These curves were calculated using ACE Mean and Chao 1 Mean estimators, recognized as the most reliable methods for assessing large communities. Additionally, estimations were made for species represented by one (singletons) or two (doubletons) individuals within the samples, following the methodology mentioned by Colwell and Coddington (1994). The underlying assumption of these estimators is that as the number of samples increases and the accumulation curves approach intersection, the inventories approach completeness (Jiménez-Valverde and Hortal 2003). All species accumulation curves were generated using the ESTIMATES ver. 9.1.0 program (Colwell and Coddington 1994).

Structure and species diversity

Rank-abundance curves were employed to evaluate the structure and composition of species within each community, facilitating the identification of dominant and rare species in each environment, following the methodology outlined by (Magurran 1998).

To quantify the diversity within each community, we utilized the Shannon-Wiener index, considering the effective number of species (Jost 2006). The true diversity value was expressed as ID = exp(H'), where ID represents the true diversity for each community, and exp(H') signifies the exponential of the Shannon index (Jost 2006).

Beta diversity (β)

To obtain the degree of similarity between species and types, we used a dendrogram (cluster) from a cluster analysis by Ward's method, which indicates, at the same time, the correlation coefficient between each type of environment (Magurran 2004).

The results obtained from the true diversity analysis allowed for comparisons of the dissimilarity in diversity between communities and the magnitude (percentage) that sets them apart from each other. To calculate the percentage of diversity dissimilarity between communities, we applied the formula (DB×100)/DA, where DA represents the diversity of community A, and DB represents the diversity of community B (Moreno 2001).

Results

We registered a total of 6 phyla, 4 subphyla,10 classes, 8 subclass, 15 orders, 15 families,18 genus, 17 species and 4 species indeterminata (Table 1). To among the four cenotes studied in Cancun, we identified 17 species. Of this species total, 38.09% (8 species) were phytoplankton, while 52.94% (9 species) were protists, including 4 species indeterminata (Fig. 3). This study contributed 11 new records for the Yucatan Peninsula, including the following species: *Euglena mutabilis*, *Lepocinclis acus*, *Phacus orbicularis*, *Coscinodiscus radiatus*, *Oscillatoria limosa*, *Arcella gibbosa*, *Amoeba radiosa* and *Mayorella vespertilioides* and the genus *Vorticella* sp., *Aspidisca* sp. and *Coleps* sp. (Table 1).

We found high completeness percentages in the inventories of all sampled cenotes, with cenote C3 standing out for achieving 100% completeness (Fig. 4). During the rainy season, we identified 12 species in C1; eight species in C3 and seven species in C4. However, no species were identified in cenote C2 cenote during this season. In contrast, during the dry season, the highest species diversity was observed in C1, where we detected four species. C2 and C3 each had two species, while C4 we had one species (Fig. 5).

The communities' structures exhibited moderate equality in C3 and C2, while a particularly high degree of equality was observed in C1 compared to the other sites. In contrast, C4 displayed relatively low equality. Overall, no dominant species were observed except for *Euglena mutabilis*, *Radiocystis geminata*, and *Aspidisca* sp., which were prominently represented in C1 and C2. However, C3 and C4 did not exhibit any dominant species (Fig. 6).

The cenote exhibiting the highest diversity, as indicated by the Shannon-Wiener index and beta diversity analysis, is C1, with a diversity index value of 2.3, followed by C3 with an index of 1.7. In contrast, the less diverse sites were C4 with a value of 1.6 and C2 with a value of 0.6. When evaluating diversity in relation to seasonality, we observed greater diversity during the rainy season, with an index of 3.2. In contrast, during the dry season, the diversity index was generally lower, with a value of 2.5 across all sites. The beta diversity analysis revealed low species similarity between cenotes, suggesting that each cenote harbors exclusive species (Fig. 7).

Table 1. List of species recorded in the study area. The species indicated with an asterisk are the new records for the Yucatan Peninsula.

Phylum Euglenophyta
Subphylum: Euglenoida
Class Euglenophyceae
Subclass Euglenophycidae
Order Euglenales
Family Euglenaceae
Genus *Euglena*
**Euglena mutabilis* F. Schmitz, 1884
Euglena texta (Dujardin) Hübner, 1886
Family Phacaceae
Genus *Lepocinclis*
**Lepocinclis acus* (O.F. Müller) B. Marin and Melkonian 2003
Genus *Phacus* Dujardin, 1841
* *Phacus orbicularis* Hübner, 1886
Phacus longicauda (Ehrenberg) Dujardin 1841
Phylum Heterokontophyta
Subphylum Ochrophytina
Class Chrysophyceae
Order Chromulinales
Family Dinobryaceae
Genus *Dinobryon*
Dinobryon sertularia Ehrenberg, 1834
Phylum Bacillariophyta
Subphylum Bacillariophytina
Class Bacillariophyceae
Subclass Coscinodiscophycidae
Order Coscinodiscales
Family Coscinodiscaceae
Genus *Coscinodiscus*
**Coscinodiscus radiatus* Ehrenberg, 1840
Subclass Bacillariophycidae
Order Thalassiophysales
Family Catenulaceae
Genus *Amphora*
Amphora ocellata Ehrenberg, 1838
Subclass Coscinodiscophycidae
Order Thalassiosirales
Family Stephanodiscaceae
Genus *Cyclotella*
Cyclotella meneghiniana Kützing, 1844
Phylum Cyanobacteriota
Class Cyanophyceae
Subclass Oscillatoriophycidae
Order Oscillatoriales
Family Oscillatoriaceae
Genus *Oscillatoria*
**Oscillatoria limosa* C. Agardh ex Gomont, 1892

Order Chroococcales
Family Microcystaceae
Genus *Radiocystis*
Radiocystis geminata Skuja, 1948

Genus *Merismopedia*
Merismopedia angularis R.H. Thompson, 1939

Order Pseudanabaenales
Family Pseudanabaenaceae
Genus *Pseudanabaena*
Pseudanabaena mucicola (Naumann & Huber-Pestalozzi) Schwabe 1964

Phylum Ciliophora
Subphylum Intramacronucleata
Class Spirotrichea
Subclass Hypotrichia
Order Euplotida
Family Aspidiscidae
*__Genus__ *Aspidisca* Ehrenberg, 1830

Class Oligohymenophorea
Subclass Peritrichia
Order Sessilida
Family Vorticellidae
*__Genus__ *Vorticella* Linnaeus, 1767

Subclass Peniculia
Order Peniculida
Family Parameciidae
Genus *Paramecium*
Paramecium aurelia Ehrenberg, 1838

Class Prostomatea
Order Prorodontida
Family Colepidae
*__Genus__ *Coleps* Nitzsch, 1827

Phylum Amoebozoa
Class Tubulinea
Order Arcellinida
Family Arcellidae
Genus *Arcella*
**Arcella gibbosa* Penard, 1890

Class Lobosa
Order Amoebida
Family Amoebidae
Genus *Amoeba*
**Amoeba radiosa* Ehrenberg, 1838

Class Discosea
Order Dermamoebida
Family Mayorellidae
Genus *Mayorella*
**Mayorella vespertilioides* Page, 1983

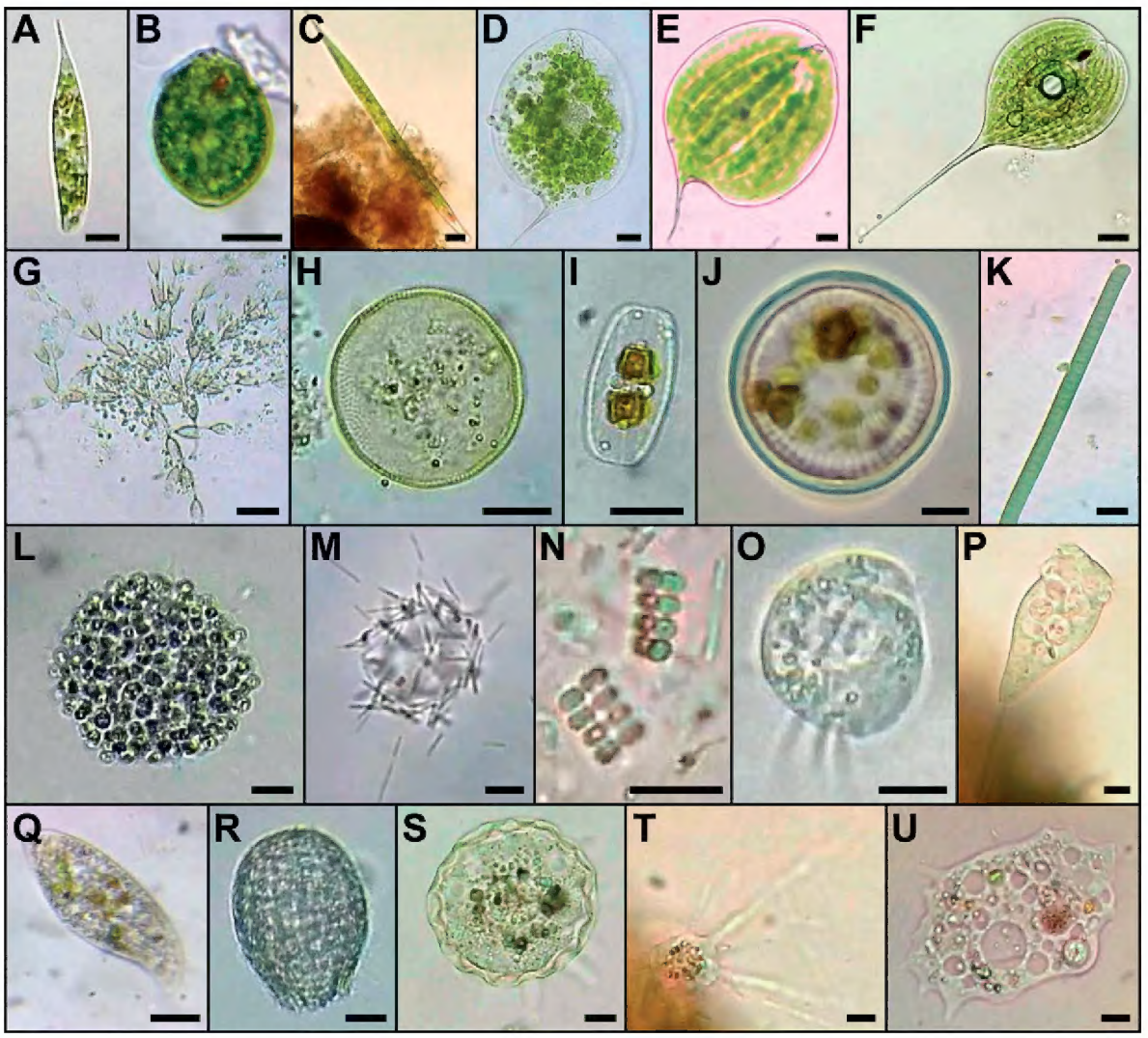

Figure 3. Species found in the sampled cenotes in Cancun, Quintana Roo, Mexico **A** *Euglena mutabilis* **B** *Euglena texta* **C** *Lepocinclis acus* **D** *Phacus* sp. **E** *Phacus orbicularis* **F** *Phacus longicauda* **G** *Dinobryon sertularia* **H** *Coscinodiscus radiatus* **I** *Amphora ocellata* **J** *Cyclotella meneghiniana* **K** *Oscillatoria limosa* **L** *Radiocystis geminata* **M** *Pseudanabaena mucicola* **N** *Merismopedia angularis* **O** *Aspidisca* sp. **P** *Vorticella* sp. **Q** *Paramecium aurelia* **F** *Coleps* sp. **S** *Arcella gibbosa* **T** *Amoeba radiosa* **U** *Mayorella vespertilioides*. Scale bars: 10 μm.

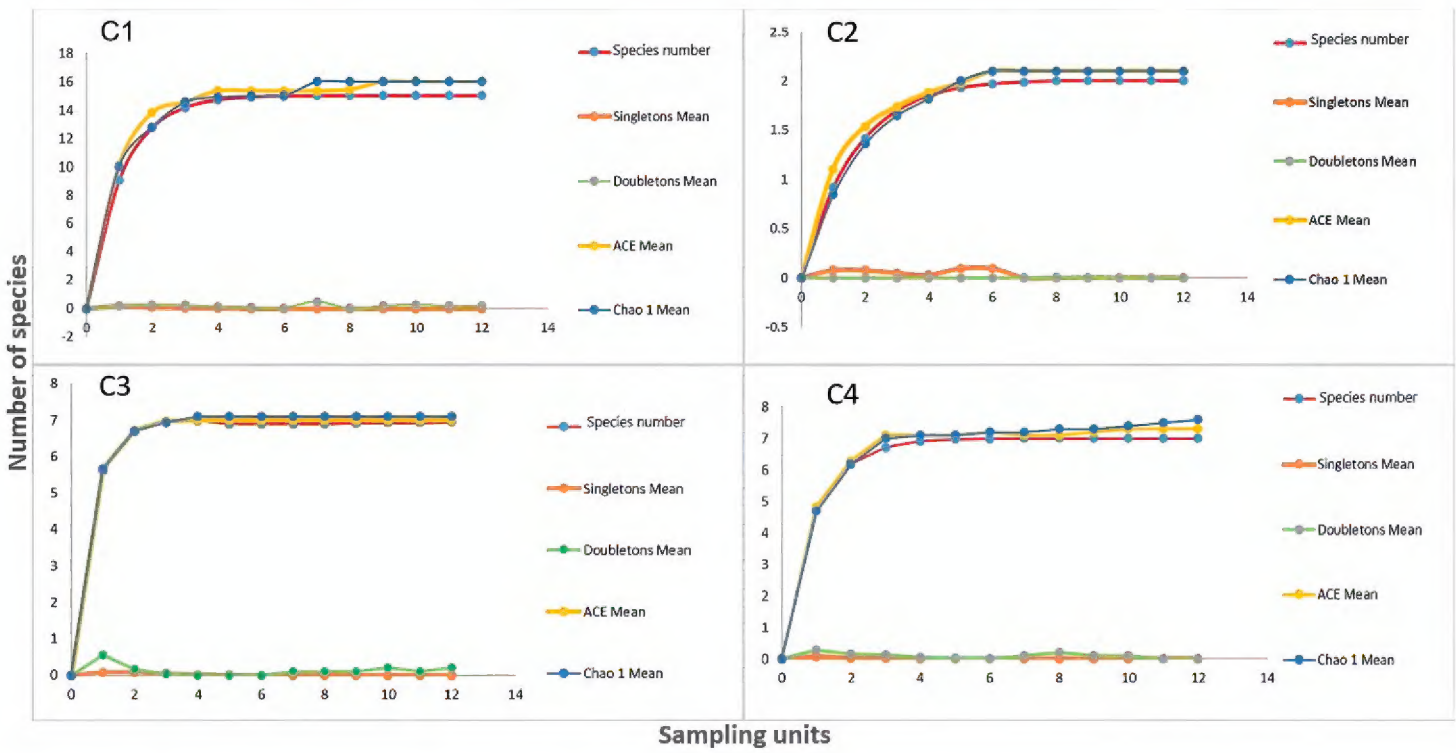

Figure 4. Completeness analysis of species inventories in analyzed cenotes using species accumulation curves and estimators.

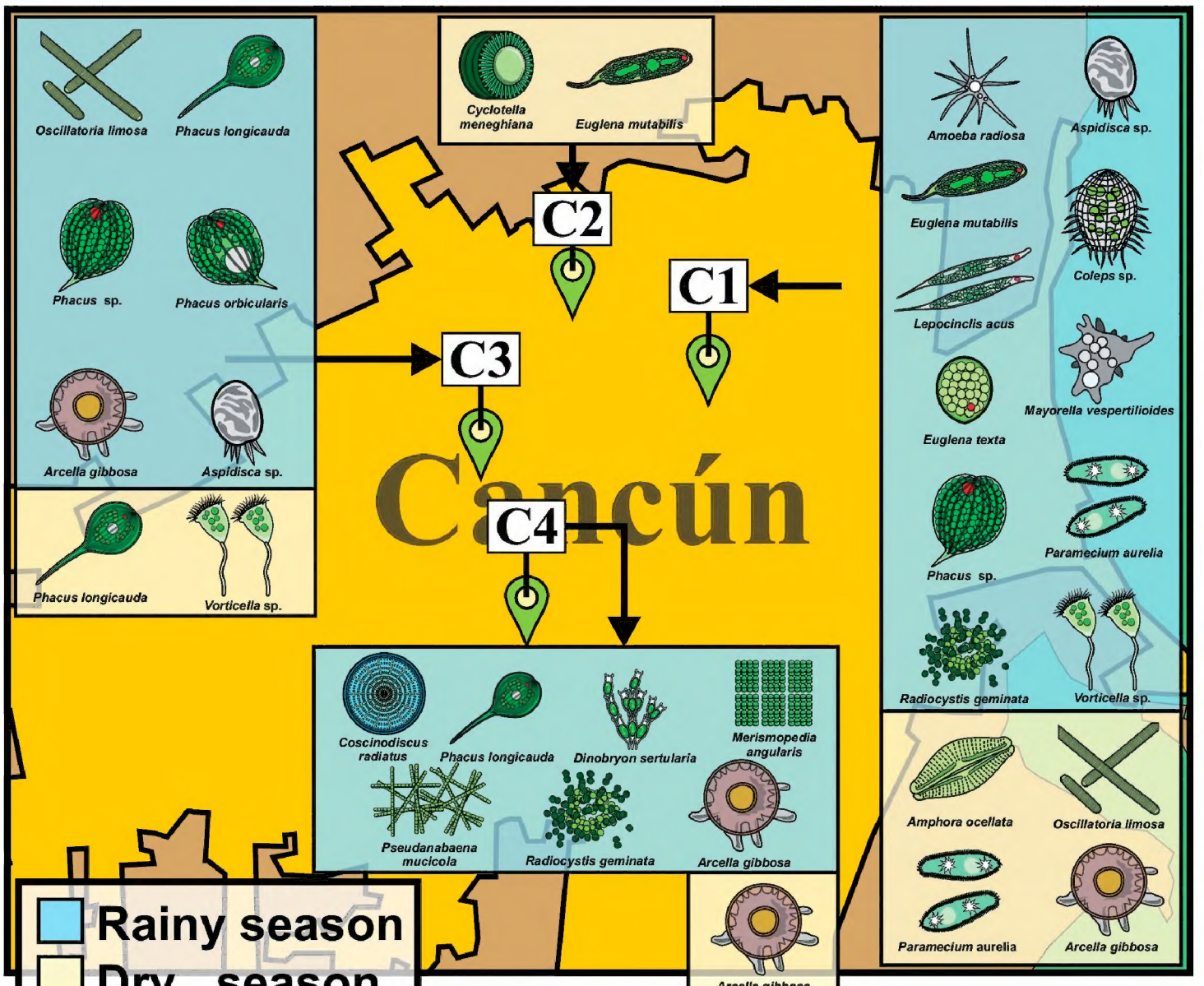

Figure 5. Sampled cenotes in Cancun, Quintana Roo, Mexico for rainy and dry seasons. The city of Cancun is highlighted in yellow. Green location markers show the sampled cenotes: C1 (Parque de los cenotes), C2 (Cenote Cauich), C3 (La Hondada), and C4 (77530 Cancun, Quintana Roo). The blue rectangles show organisms sampled in the rainy season and the light-yellow rectangles show organisms sampled in the dry season.

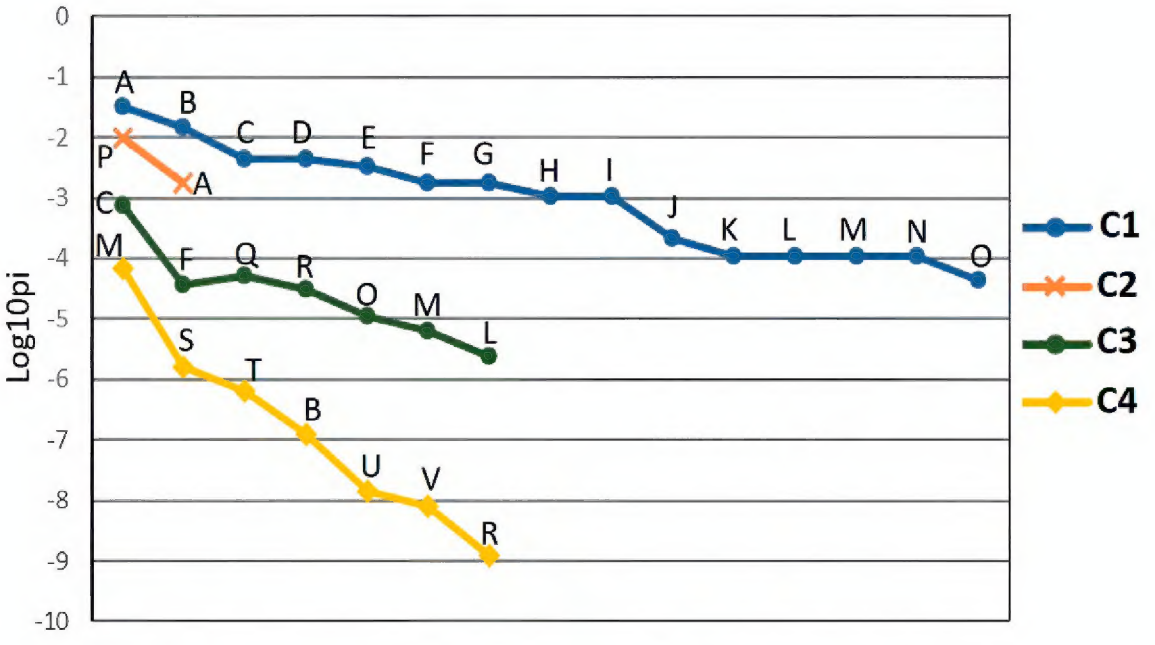

Figure 6. Rank-abundance curves of phytoplankton and protists communities in the 4 sampled urban cenotes in Cancun. C1 corresponds to the abundance found in cenote 1, C2 shows the abundance found in cenote 2, C3 indicates the abundance found in cenote 3, and C4 shows abundance found in cenote 4.

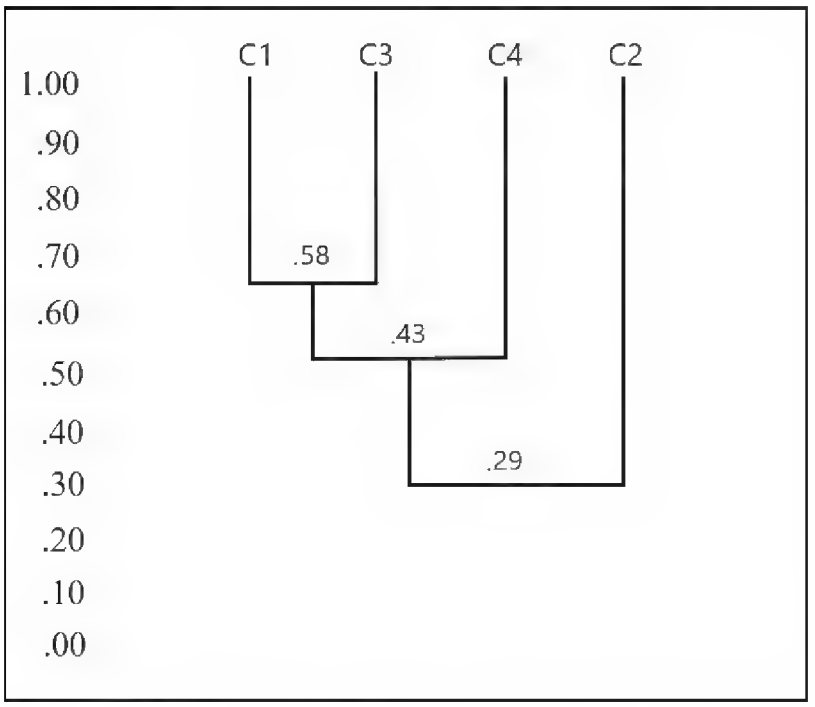

Figure 7. Cluster analysis for each cenote and between cenotes. The analysis shows the diversity similarity found in each cenote and between the different types of habitats analyzed in each urban cenote.

Discussion

The loss of tropical biodiversity has become a growing concern due to the rapidly expanding human population and the increasing demand for resources such as land and water for various habitats (Edwards et al. 2019). Preserving these delicate ecosystems necessitates studies that underscore the urgency of their conservation efforts. For instance, the biodiversity of the coastal ecosystems along México's Gulf and Atlantic coasts faces threats from various anthropic activities. In the Mexican Caribbean, the recent surge in human population has resulted in escalated environmental impacts on both freshwater and marine environments (Guerra-Castro et al. 2020). Despite these challenges, the biodiversity of freshwater and marine ecosystems in the Mexican Caribbean remains poorly studied, resulting in a significant knowledge gap regarding these aquatic systems and their phytoplankton and protists communities. Cenotes, as heterotrophic systems, play a unique role due to the microorganisms' ability to balance the microecosystem, giving rise to a distinct cenote ecosystem (Schmitter-Soto et al. 2002a). While studies have focused on the taxonomic composition of phytoplankton in coastal lagoons in the Yucatan Peninsula (Herrera-Silveira et al. 1999; Nava-Ruiz and Valadez 2012; Valadez et al. 2013), there is a notable absence of references regarding the structure of phytoplankton and protists communities in urban cenotes within the Mexican Caribbean. This study illuminates the richness in the structure and composition of species within cenotes associated with urban regions, which today dominate the landscape. These cenotes hold ecological significance, and their diversity serves as a crucial indicator of ecosystem health.

While the sampling completeness in each of the cenotes is relatively high, hovering around 85%, it is essential to acknowledge that achieving comprehensive representation of microbial species in any given environment is a formidable challenge. Studies aiming to assess species diversity strive to gain a holistic understanding of a site's diversity, but achieving complete representation is often an elusive goal (Hortal et al. 2006), as demonstrated

in our study. Several factors contribute to the completeness of the inventories, including sampling bias, methodologies employed for sampling (such as trapping techniques), the timing of sampling, fluctuations in environmental conditions, and the structural complexity of the ecosystem under investigation (Hortal et al. 2006). Nonetheless, it's important to note that a representative sample obtained through systematic sampling can still provide a valuable reflection of a site's diversity (Moreno et al. 2011). Interestingly, our study revealed a higher abundance of protists compared to phytoplankton organisms during the rainy season as opposed to the dry season (Fig. 5). This finding contrasts with the observations of Kouassi et al. (2013) in the Adzopé Reservoir, located in the city of the Adzopé, Ivory Coast, Africa, where they noted greater species abundance during the dry season and fewer species in the rainy season. It's important to note that these differences can be attributed to the distinct characteristics of the two ecosystems. Furthermore, studies conducted in coastal lagoons of the Yucatan Peninsula (Herrera-Silveira et al. 1999; Nava-Ruiz and Valadez 2012) found no significant differences in species richness between seasons but observed variations in dominant species throughout the year. These differences likely stem from the varying tolerance levels and physiological characteristics of individual organisms, as well as the unique environmental conditions in each site.

We identified four phyla of phytoplankton, with five species belonging to Euglenophyta and one species indeterminate, one species to Heterokontophyta, three species to Bacillariophyta and four species to Cyanobacteriota. Notably, Euglenozoa emerged as the most diverse group among them. It's worth highlighting that many of these species represent the first documented records for the Mexican Caribbean (Luna-Pabello 2006; Sigala-Regalado et al. 2016; Quiroz-González and Rivas-Acuña 2017; Guiry and Guiry 2020; WoRMS 2020) (Table 1). Seasonal precipitation has been a crucial factor correlated with an increase in phytoplankton biomass in various aquatic environments (Okoth et al. 2009). This phenomenon has significantly contributed to the heightened production and diversity of phytoplankton and protists species during the rainy season, a trend not previously reported for cenotes in the northern Yucatan Peninsula. Furthermore, Troccoli et al. (2004) observed a relationship between hydrographic variables and phytoplankton blooms in coastal areas along the beaches of Campeche, Yucatan, and Quintana Roo. Their analysis of the three coastal zones suggests that the differences in hydrology and biology between Campeche and Yucatan/Quintana Roo were attributed to marine currents. In cenotes, underground currents play a significant role, so it's crucial to consider variables such as nutrient concentrations, temperature, and various physical parameters as potential drivers of biodiversity.

In our study, a higher density of protists was observed in sampled cenotes compared to phytoplankton. We identified two phyla: Ciliophora with one species and three species indeterminata and Amoebozoa with three organisms. Remarkably, all species, except for *P. aurelia*, represented the first record instances for the Mexican Caribbean region, contributing to an increase in species diversity in the northern Yucatan Peninsula. Furthermore, we observed a higher density of protists during the rainy season, particularly in cenote C1. This finding aligns with the observations of Sigala-Regalado et al. (2011), who emphasized the importance of protists in ecosystems while

nothing their limited study in cave environments. Sigala-Regalado et al. (2011) reported eight species of ciliates, three species of flagellates, and one amoeboid species within cave systems in Queretaro, Mexico, over a year. Five of these species were reported for the first time inside cave systems, and an additional three species are new records for caves. In our report, we identified four species and three species indeterminata of which six representing the first recorded instances for the Mexican Caribbean. This variation suggests that each cenote harbors unique species, contributing to low species similarity between the analyzed sites, as demonstrated in the cluster analysis. This pattern is consistent with the observations made by Sigala-Regalado et al. (2011), who noted that each ecosystem or habitat tends to host distinct species with few shared species. Protists exhibit a wide range of dietary requirements (Pratt and Cairns 1985), and these requirements were met by the different cenotes. Additionally, the broad environmental tolerances of common taxa, such as *Aspidisca* sp., *Vorticella* sp. and *P. aurelia*, suggest that these species could potentially be found in nearly every natural system.

Until now, comprehensive studies evaluating the structure and diversity of communities in urban cenotes have been notably lacking. This represents a significant gap in our understanding, as the mere presence of species does not provide insight into the overall quality and ecological health of aquatic systems. Therefore, this work serves as a crucial foundational step for future research endeavors aimed at assessing the richness, abundance, and structural characteristics of these communities. These organisms, occupying the primary trophic level within the ecosystem, play a fundamental role in shaping the entire food web. Consequently, ongoing, and systematic monitoring efforts are imperative to gauge and ensure the health and sustainability of these vulnerable ecosystems.

Conclusions

We identified eight species of phytoplankton and one species indeterminata while, nine species of protists and three species indeterminata in the cenotes of Cancun, Quintana Roo, Mexico. Some of these species represent new records, underscoring the complexity and diversity of these ecosystems. Given the ecological significance of cenotes and their vital role in the economic sustenance of the region, it is imperative to implement effective management and conservation strategies. This is crucial in order to mitigate the potential polluting factors resulting from current cenotes management practices.

Furthermore, compounding the existing challenges, there is currently a lack of precise knowledge regarding the behavior and dynamics of these urban aquifers. This deficiency in information severely hinders effective management strategies to mitigate potential future negative impacts. There exists a notable gap in studies providing comprehensive inventories and characterization of urban cenotes, which are essential for informed decision-making in their management, conservation, and sustainable utilization. Addressing this issue necessitates the implementation of public policies and actions, coupled with technical and scientific support from hydrological systems. Furthermore, active participation from society is vital to collectively protect and conserve these ecosystems.

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Supplementary material 1

Kinds of cenotes sampled

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Data type: tiff

Explanation note: Kinds of cenotes sampled (A) C1 (Parque de los cenotes) a mix between cavern and aguada-type cenote; (B) C2 (Cenote Cauich) a grotto or a pitcher-type cenote; (C) C3 (La Hondada) an aguada-type cenote; and (D) C4 (77530 Cancún, Q. Roo) an aguada-type cenote.

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